Miltenyi Biotec

CD4$^+$CD62L$^+$ T Cell Isolation Kit
mouse

Order no. 130-106-643

Contents

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

1. Description

This product is for research use only.

Components	**1 mL CD4$^+$ T Cell Biotin-Antibody Cocktail, mouse:** Cocktail of biotin-conjugated monoclonal anti-mouse antibodies against CD8a, CD11b, CD11c, CD19, CD25, CD45R, CD49b, CD105, Ter-119, MHC class II, and TCRγ/δ. **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1). **2 mL CD62L (L-selectin) MicroBeads, mouse:** MicroBeads conjugated to monoclonal anti-CD62L antibodies (isotype: rat IgG2a).
Capacity	For 10^9 total cells.
Product format	All components are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of the MACS® Separation

The isolation of CD4$^+$CD62L$^+$ cells is performed in a two-step procedure. First, the non-CD4$^+$ cells are indirectly magnetically labeled with a cocktail of biotin-conjugated antibodies, as primary labeling reagent, and anti-biotin monoclonal antibodies conjugated to MicroBeads, as secondary labeling reagent. In between the two labeling steps no washing steps are required. The labeled cells are subsequently depleted by separation over a MACS® Column, which is placed in the magnetic field of a MACS Separator.
In the second step, the CD4$^+$CD62L$^+$ T cells are directly labeled with CD62L (L-selectin) MicroBeads and isolated by positive selection from the pre-enriched CD4$^+$ T cell fraction by separation over a MACS Column, which is placed in the magnetic field of a MACS Separator.
After removing the column from the magnetic field, the magnetically retained CD4$^+$CD62L$^+$ T cells can be eluted as the positively selected cell fraction.

Mouse splenocytes: Depletion of non-CD4$^+$ T cells

1. Indirect magnetic labeling of non-CD4$^+$ T cells with CD4$^+$ T Cell Biotin-Antibody Cocktail and Anti-Biotin MicroBeads.
2. Magnetic separation using an LS Column.

Pre-enriched CD4$^+$ T cells (flow-through fraction): Positive selection of CD4$^+$CD62L$^+$ T cells

1. Direct magnetic labeling of CD4$^+$CD62L$^+$ T cells with CD62L (L-selectin) MicroBeads.
2. Magnetic separation using a MS Column.

CD4$^+$CD62L$^+$ T cells (eluted fraction)

1.2 Background information

The CD4$^+$CD62L$^+$ T Cell Isolation Kit has been developed for the isolation of CD4$^+$CD62L$^+$ T helper cells from spleen and lymph nodes.
CD62L (L-selectin) is highly expressed on naive T cells and downregulated upon activation. It is also expressed on a small subset of memory T helper cells, the central memory T cells, which can be distinguished from naive T helper cells by their high expression of CD44. Furthermore, CD62L is expressed on most thymocytes, naive CD8$^+$ T cells, B cells, dendritic cells, macrophages, NK cells, neutrophils, eosinophils, regulatory T cells, and TCRγ/δ$^+$ T cells.
For isolation of CD4$^+$CD62L$^+$ T helper cells, the non–T helper cells as well as regulatory T cells and TCRγ/δ$^+$ T cells are depleted by indirect magnetic labeling using a cocktail of lineage-specific biotin-conjugated antibodies against CD8a, CD11b, CD11c, CD19, CD45R, CD49b, CD105, MHC class II, and Ter-119, as well as antibodies against CD25 and TCRγ/δ in combination with Anti-Biotin MicroBeads.
Subsequently, CD4$^+$CD62L$^+$ T cells are positively selected from the enriched CD4$^+$ T helper cell fraction with CD62L (L-selectin) MicroBeads.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

140-004-774.03

1.3 Applications

- Isolation of $CD4^+CD62L^+$ T cells from single cell suspensions of spleen and lymph nodes for further phenotypical or functional characterization.

1.4 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: Depletion of non-$CD4^+$ T cells can be performed on an LS Column. The subsequent positive selection of $CD4^+CD62L^+$ T cells can be performed on a MS Column. Depletion and positive selection can also be performed by using the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
autoMACS	2×10^8	4×10^9	autoMACS Pro

▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ or SuperMACS™ II Separators. For details refer to the respective MACS Separator data sheet.

- (Optional) gentleMACS™ Dissociator (130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427).
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD44-FITC, CD3ε-APC-Vio®770, CD4-VioBlue®, CD25-APC, CD45-VioGreen™, or CD62L-PE. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

When working with lymphoid organs, non-lymphoid tissues, or peripheral blood, prepare a single-cell suspension using manual methods or the gentleMACS Dissociators.

For details refer to www.gentleMACS.com/protocols.

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).

2.2 Magnetic labeling of non-$CD4^+$ T cells

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to 10^8 total cells. When working with fewer than 10^8 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^8 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters (30 µm) # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 400 µL of buffer per 10^8 total cells.
4. Add 100 µL of $CD4^+$ T Cell Biotin-Antibody Cocktail per 10^8 total cells.
5. Mix well and incubate for 5 minutes in the refrigerator (2–8 °C).
6. Add 300 µL of cold buffer and 200 µL of Anti-Biotin MicroBeads per 10^8 total cells.
7. Mix well and incubate for additional 10 minutes in the refrigerator (2–8 °C).
8. Proceed to magnetic separation (2.3).

2.3 Magnetic separation: Depletion of non-$CD4^+$ T cells

▲ Always wait until the column reservoir is empty before proceeding to the next step.

Magnetic separation with LS Columns

1. Place LS Column in the magnetic field of a suitable MACS Separator. For details refer to the LS Column data sheet.
2. Prepare column by rinsing with 3 mL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched $CD4^+$ T cells.
4. Wash column with 3 mL of buffer. Collect unlabeled cells that pass through, representing the enriched $CD4^+$ T cells, and combine with the flow-through from step 3.

5. (Optional) Remove column from the separator and place it on a suitable collection tube. Pipette 5 mL of buffer onto the column. Immediately flush out the magnetically labeled non-$CD4^+$ T cells by firmly pushing the plunger into the column.
6. Proceed to 2.4 for the labeling of $CD4^+CD62L^+$ T cells.

Depletion with the autoMACS® Pro Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS® Pro Separator.

▲ Buffers used for operating the autoMACS Pro Separator should have a temperature of ≥10 °C.

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose the following program:

   Depletion: Depletes
   Collect negative fraction in row B of the tube rack.
4. Proceed to 2.4 for the labeling of $CD4^+CD62L^+$ T cells.

2.4 Magnetic labeling of $CD4^+CD62L^+$ T cells

▲ Volumes for magnetic labeling given below are for an initial starting cell number of up to 10^8 total cells. For higher initial cell numbers, scale up all volumes accordingly.

1. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 800 µL of buffer.
3. Add 200 µL of CD62L (L-selectin) MicroBeads.
4. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
5. Wash cells by adding 10 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
9. Resuspend up to 10^8 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
10. Proceed to magnetic separation (2.5).

2.5 Magnetic separation: Positive selection of $CD4^+CD62L^+$ T cells

Positive selection with MS Columns

1. Place MS Column in the magnetic field of a suitable MACS Separator. For details refer to the MS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 2×500 µL of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3.

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.

   ▲ **Note:** To perform a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of a collection tube.
6. Pipette 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

Positive selection with the autoMACS® Pro Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS® Pro Separator.

▲ Buffers used for operating the autoMACS Pro Separator should have a temperature of ≥10 °C.

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose the following program:

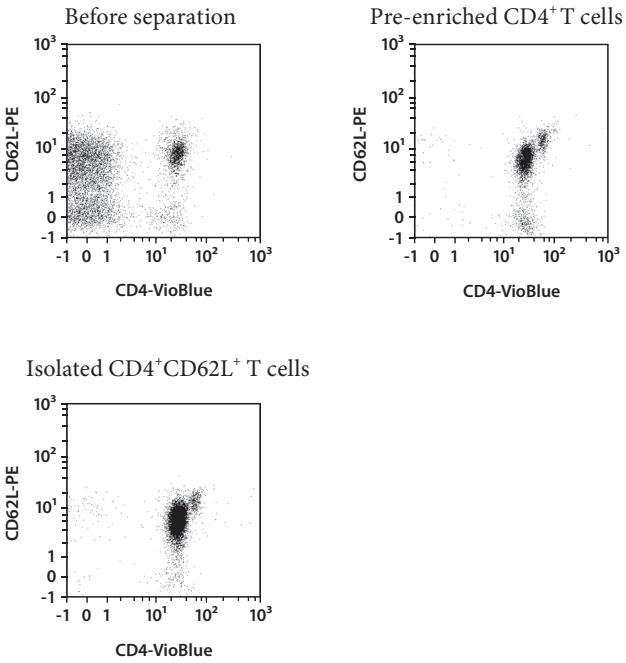
   Positive selection: Possel
   Collect positive fraction in row C of the tube rack. This is the enriched $CD4^+CD62L^+$ cell fraction.

140-004-774.03

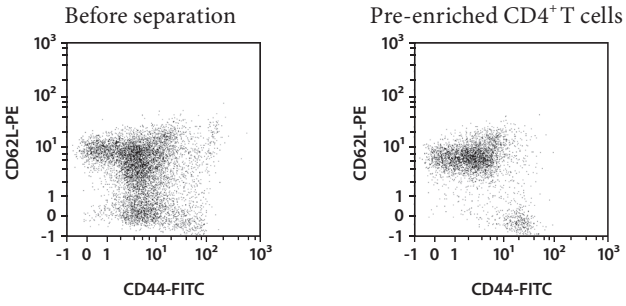
3. Example of a separation using the CD4$^+$CD62L$^+$ T Cell Isolation Kit

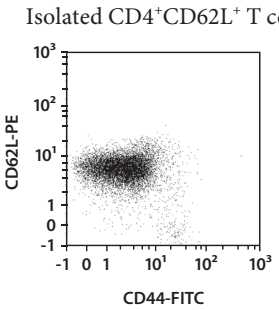
CD4$^+$CD62L$^+$ cells were isolated from mouse spleen by using the CD4$^+$CD62L$^+$ T Cell Isolation Kit, an LS and an MS Columns, a MidiMACS™ and a MiniMACS™ Separator. The cells were fluorescently stained with CD45-VioGreen, CD4-VioBlue, and CD62L-PE for detection of naive T cells (A), as well as with CD62L-PE and CD44-FITC for detection of central memory T cells (B). Additionally, cells were stained with CD25-APC to illustrate the removal of CD25$^+$CD62L$^+$ regulatory T cells (C). Analysis was performed for CD45$^+$ cells by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

A) Detection of naive CD4$^+$ T cells

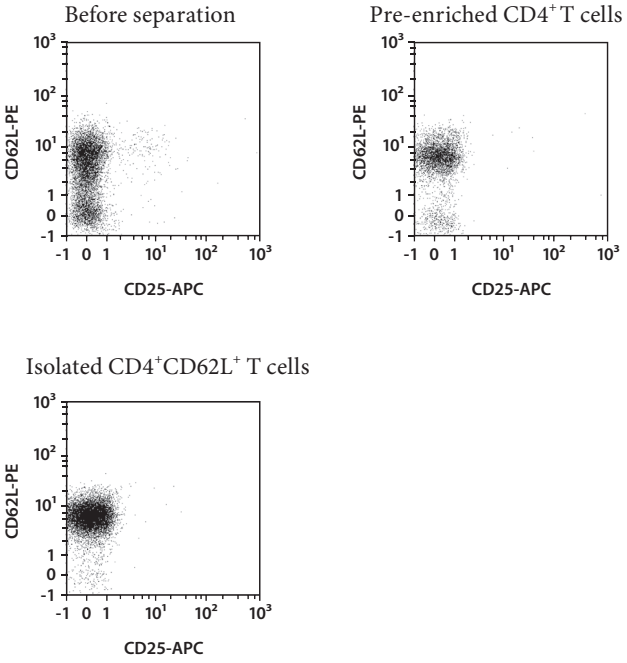


B) Detection of naive T cells





C) Removal of regulatory T cells



Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

140-004-774.03

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

autoMACS, gentleMACS, MACS, MACSQuant, MidiMACS, the Miltenyi Biotec logo, MiniMACS, OctoMACS, QuadroMACS, SuperMACS, VarioMACS, Vio, VioBlue, and VioGreen are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide.